PATENT ABSTRACTS OF JAPAN

(11)Publication number : **10-191961**
(43)Date of publication of application : **28.07.1998**

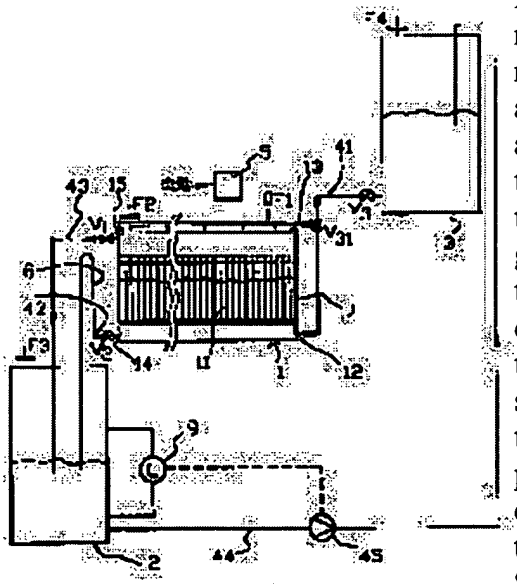
(51)Int.CI. C12M 3/00
C12N 5/06

(21)Application number : **09-160316**
(22)Date of filing : **17.06.1997**

(71)Applicant : **LIAU MING-YI**
(72)Inventor : **LIAU MING-YI**

(30)Priority
Priority number : **96 85107344** Priority date : **18.06.1996** Priority country : **TW**

(54) CULTURING OF CELL AND CULTURING APPARATUS



(57)Abstract:
PROBLEM TO BE SOLVED: To provide a method for culturing cells by which removal of a metabolite and exchange of a culturing medium are readily performed, the surface of a cell attachment substrate is dipped in or exposed to the culturing medium, the supply of a nutrient by the culturing medium and the supply of a fresh gas are easily performed and thereby facilitating the application to a high density cell, a sufficient dissolved oxygen for cell growth is supplied by the culturing medium without causing a high sheer force, a sudden flaking off of the cells from the cell substrate is avoided, control is readily performed and a production scale is easily enlarged, and further to provide an apparatus therefor.
SOLUTION: This culturing apparatus has a cell culturing room 11 having charging and discharging means 14, 13 and 12, and a circulating pump 45 for circulating a culturing medium through the charging and discharging means 14, 13 and 12 to pass the cell culturing room 11. The level of the culturing medium against the surface of the substrate 7 is changed upper or lower between a level higher than the upper end height of the substrate 7 and a level lower than the lower end of the substrate 7 by the circulating pump 45 so that the culturing medium may be supplied when the level is decreased to the lower level and the culturing medium is discharged when the level is increased to the higher level within the state in which the

substrate 7 is not moved in the cell culturing room 1.

LEGAL STATUS

[Date of request for examination]	17.06.1997
[Date of sending the examiner's decision of rejection]	
[Kind of final disposal of application other than the examiner's decision of rejection or application converted registration]	
[Date of final disposal for application]	
[Patent number]	2980867
[Date of registration]	17.09.1999
[Number of appeal against examiner's decision of rejection]	
[Date of requesting appeal against examiner's decision of rejection]	
[Date of extinction of right]	

CLAIMS

[Claim(s)]

[Claim 1] The cell culture room which has an appearance means (14) and an entering means (13 12) (11), Two or more base materials for cell adhesion formed in the cell culture room (11) (7), The circulation means through which is opened for free passage with an appearance means (14) and an entering means (13 12), and comes out of a culture medium, and a cell culture room (11) is made to circulate through a means (14) and an entering means (13 12) (2, 3, V3 and V2, 6, 8, 44, 45), In the culture apparatus of a preparation **** cell, said circulation means in the condition of not moving a base material (7) to a cell culture room (11) Raising and lowering actuation are carried out for the level of the culture medium to the front face of a base material (7) between level higher than the upper limit marginal height (h2) of a base material (7), and level lower than lower limit marginal height (h1). And the culture apparatus of the cell characterized by what is constituted so that it may come out when it will enter if the level of a culture medium falls on said low level, and a culture medium is supplied via a means (12 13) and the level of a culture medium goes up to said high level, and a culture medium can be discharged via a means (14).

[Claim 2] Said circulation means is the culture apparatus of the cell according to claim 1 characterized by what it has for the 1st tank (2) which is open for free passage with an appearance means (14), and is put on the place lower than the pars basilaris ossis occipitalis of a cell culture room (11).

[Claim 3] Said circulation means is the culture apparatus of the cell according to claim 2 characterized by what it has for the 2nd tank (3) put on the location higher than head

lining of a cell culture room (11) by the upstream of a cell culture room (11).
[Claim 4] Said circulation means is the culture apparatus of the cell according to claim 3 characterized by what it has for the pump means (45) established between the 1st tank (2) and the 2nd tank (3).
[Claim 5] Said circulation means is the culture apparatus of the cell according to claim 4 characterized by what it has for the siphon means which will suck up if it is open for free passage with each of an appearance means (14) and the 1st tank (2) and the level of a culture medium goes up to said high level, and acts.
[Claim 6] Said circulation means is the culture apparatus of the cell according to claim 5 characterized by what it came out so that the flow of a culture medium might be controlled, and it has for the 1st control valve (V2) currently opened for free passage and installed in the means (14), and the 2nd control valve (V3) which enters so that the inflow of a culture medium may be controlled, and is opened for free passage and installed in the means (12 13).
[Claim 7] Said circulation means is the culture apparatus of the cell according to claim 6 characterized by what it has the cell culture room (11), and the overflow pipe (43) which was open for free passage and the overflow valve (V1) prepared in this overflow pipe (43) for in the part higher than the top of a base material (7).
[Claim 8] Said circulation means is the culture apparatus of the cell according to claim 7 characterized by what it has a culture-medium surface level measurement means (9) to measure the level of the front face of the culture medium in the 1st tank (2) for.
[Claim 9] Said entering means is the culture apparatus of the cell according to claim 8 characterized by what it has for the 1st inlet port (13) established in the part near head lining of a cell culture room (11), and the 2nd inlet port (12) established in the part near the pars basilaris ossis occipitalis of a cell culture room (11).
[Claim 10] The culture apparatus of the cell according to claim 9 characterized by what it has further a culture-medium homogeneity distribution means (8) to distribute to homogeneity the upper part of the base material (7) in a cell culture room (11), or the culture medium which is prepared caudad and flows into a cell culture room (11) for.
[Claim 11] The culture apparatus of the cell according to claim 10 characterized by what it has further a gas supply means (5) to supply a gas to a cell culture room (11) from the upper part of a cell culture room (11) for.
[Claim 12] (a) The cell culture room (11) which has . appearance means (14) and an entering means (13 12), and a base material for cell adhesion (7) is installed.;
(b) A culture medium is supplied to . cell culture room (11), and a cell is made to adhere to a base material (7).;
(c) in the condition of not moving a base material (7) to a cell culture room (11), growing up a cell into the front face of . base material (7) While circulating a culture medium via an appearance means (14) and an entering means (13 12), taking-up-and-down actuation of the level of the culture medium to the front face of a base material (7) is carried out between level higher than the upper limit marginal height (h2) of a base material (7), and level lower than lower limit marginal height (h1).;
(d) It controls to come out, if the level of . culture medium goes up to said high level, and to make a culture medium flow out via a means (14).;
(e); controlled to enter if the level of a culture medium falls on said low level, and to

make a culture medium flow via a means (12 13) -- the culture approach of the cell characterized by things.

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] Especially this invention relates to the culture approach of an anchorage dependency monolayer cell, and a culture apparatus about the culture approach of an animal cell, and a culture apparatus.
[0002]
[Description of the Prior Art] Development of the culture approach of an animal cell is indispensable indispensable work of the medicine manufacture industry with the increment in need of an animal cell origin chemical. And since a cell suspends in a culture medium and it cannot grow up when cultivating many animal cells, such as a mammals animal cell, the base material as a scaffold must be made to carry out adhesion immobilization of the cell. Many culture methods of such an anchorage dependency animal cell are developed until now. For example, the micro carrier in a tissue culture machine, a roller bottle type, a cell culture machine, and a mixing vessel, a hollow fiber, a pottery method, etc. are so.
[0003] [Wei-Shou Hu et al and "Animal Cell Bioreactors-Recent Advances and Challenges to Scale-up" The Candian Journalof Chemical Engineering; "Specialized Techniques" Volume 69 April and 1991, page 409-420"; "Specialized Techniques" Culture of Animal Cells Chapter 23 pages 371-377].
[0004] In the process of animal cell culture, the basic demand fully supplies a gas and nutrient, such as oxygen. Growth of a cell is checked if a gas or nutrient is not fully supplied. And control of the level of the amount of metabolite is also required. It is because growth of a cell will be checked if the metabolite concentration in a culture medium is too high.
[0005] Said roller bottle type is a bus mold, and the circulation network of a culture medium does not attach it. This method is equipped with the bottle which holds a culture medium, and bearing is carried out on a frame, and rolls. Usually, while making a cell adhere to the wall of a bottle and making it grow up, putting the double ground of 1 / 10 - 1/5 of the inner capacity of a bottle into one of the bottle of this, and rotating a bottle, the gas exchange between a gaseous phase and the liquid phase is promoted at the time of rotation, and it makes the amount of dissolved oxygen in a culture medium increase with the thin film formed in the bottle wall front face which is not dipped in a culture medium. Therefore, even if the consistency of a cell becomes high depending on this method, supply of gases, such as oxygen, can fully be received, and the adhesion surface area of the cell to a bottle wall will increase, and there will be a loose stirring effect. And in this method, a fresh culture medium can also be added by injectors and distribution apparatus, such as a pipet or a peristalsis mold pump.
[0006] However, depending on this method, pH and dissolved oxygen are uncontrollable. Since the circulation network of a culture medium moreover does not stick and exclusion of harmful nature metabolite and the supplement of nutrient must be carried out by exchanging culture media frequently, it takes time and effort. And since many bottles

must be used for mass production, time and effort is taken more and uniform quality is also difficult to get. Therefore, there is a limitation in the application range.
[0007] The cell culture method which the culture-medium circulation network attaches is advantageous to the supplement of a culture medium, and exclusion of metabolite. In such a method, it is always immersed in the culture medium, and the circulation velocity of a culture medium is controlled, and, as for a cell, nutrient and dissolved oxygen are fully supplied.
[0008] Although a micro carrier method is also a cycloid type and expansion of mass production can be made easy, since cost is not only high, but it is easy to separate a cell from a micro base material, actuation is inconvenient when especially shearing force is large.
[0009] Another cycloid type culture method is the bioreactor of plug flows, such as a hollow fiber or a pottery method. In a hollow fiber method, a culture medium passes along the lumen of fiber by the high rate of flow, and only a pole part permeates the fiber film. Although this hollow fiber method is a method with high culture consistency and effectiveness, since supply of the nutrient of small molecular weight and exclusion of metabolite are attained by the diffusion for which it usually depends on the concentration gradient which crosses the film, dissolved oxygen and nutrient run short with the increment in the die length of a hollow fiber, or thickness. Therefore, it is difficult for the mass production of a cell too in a hollow fiber method.
[0010] And in a pottery method, while the cell is inoculated into the path of a porous ceramic cylinder and a culture medium supplies nutrient, it passes through said path so that metabolite may be removed. In order to make the amount of dissolved oxygen increase at this time, a culture medium is circulated with a pump. However, if the circulation velocity of a culture medium is made to increase in order to supply sufficient amount of dissolved oxygen, a cell may exfoliate from a scaffold (fixed front face) by the increment in the circulation velocity of this culture medium, and a bad influence may be caused to productivity.
[0011]
[Problem(s) to be Solved by the Invention] It is offering the culture approach of a cell and culture apparatus which this invention is made under the above-mentioned situation, and the purpose of this invention can perform removal of metabolite, and exchange of a culture medium easily, and the scaffold front face of a cell surfaces from a culture medium, and the gas exchange between the thin film of a culture medium and a gas can carry out easily, and can solve the problem oxygen's being insufficient, also in a high density cell even if.
[0012] This invention aims at offering the culture approach of a cell and culture apparatus which can fully supply the dissolved oxygen for growth of a cell by the culture medium again, without high shearing force occurring.
[0013] This invention aims at being able to control easily and the scale-up of a production scale offering the easy culture approach of a cell and an easy culture apparatus further again.
[0014] Furthermore, the adhesion surface area of a cell is large, and this invention aims to let it offer the culture approach of a cell and culture apparatus which can exfoliate the cell from culture-medium material easily.
[0015]

[Means for Solving the Problem] In order that this invention may attain the above-mentioned purpose, in the culture apparatus of the cell of this invention The cell culture room which has an appearance means and an entering means, and the base material for cell adhesion formed in the cell culture interior of a room, In the culture apparatus of the cell equipped with the circulation means through which is opened for free passage with an appearance means and an entering means, and comes out of a culture medium, and a cell culture room is made to circulate through a means and an entering means, a circulation means in the condition that a base material does not move to a cell culture room in the first half Between level higher than the upper limit marginal height of a base material, and level lower than lower limit marginal height, raising and lower and the level of the culture medium to the front face of a base material is operated. And it is constituted so that it may come out when it will enter if the level of a culture medium falls on said low level, and a culture medium is supplied via a means and the level of a culture medium goes up to said high level, and a culture medium can be discharged via a means.
[0016] The 1st tank which said circulation means is open for free passage with an appearance means preferably, and is put on the place lower than the pars basilaris ossis occipitalis of a cell culture room, The 2nd tank put on the location higher than head lining of a cell culture room by the upstream of a cell culture room, The pump means established between the 1st tank and the 2nd tank, and the siphon means which will suck up if it is open for free passage with each of an appearance means and the 1st tank and the level of a culture medium goes up to said high level, and acts, The 1st control valve which comes out so that the flow of a culture medium may be controlled, and is opened for free passage and installed in the means, The 2nd control valve which enters so that the inflow of a culture medium may be controlled, and is opened for free passage and installed in the means, It has a culture-medium surface level measurement means to measure the level of a cell culture room, the overflow pipe which was open for free passage, the overflow valve prepared in the overflow pipe, and the front face of the culture medium in the 1st tank etc., if needed from the part higher than the top of a base material.
[0017] Moreover, the entering means is equipped with the 1st inlet port preferably established in the part near head lining of a cell culture room, and the 2nd inlet port established in the part near the pars basilaris ossis occipitalis of a cell culture room. And the culture apparatus of the cell of this application is further equipped with a culture-medium homogeneity distribution means to distribute to homogeneity the upper part of the base material of the cell culture interior of a room, or the culture medium which is prepared caudad and flows into a cell culture room preferably, and a gas supply means to supply a gas to a cell culture room from the upper part of a cell culture room again.
[0018] In order to attain the purpose of this invention mentioned above, by the culture approach of the cell of the invention in this application And a :(a). appearance means and an entering means, In the condition of not moving a base material to a cell culture room, installing the cell culture room which has a base material for cell adhesion, supplying a culture medium to a;(b). cell culture room, making a cell adhering to a base material, and growing up a cell into the front face of a;(c). base material While circulating a culture medium via an appearance means and an entering means So that it will come out if taking-up-and-down actuation of the level of the culture medium to the front face of a base material is carried out between level higher than the upper limit marginal height of a

base material, and level lower than lower limit marginal height and the level of a;(d). culture medium goes up to said high level, and a culture medium may be made to flow out via a means ; which is controlled and is controlled to enter if the level of; culture medium falls on said low level, and to make a culture medium flow via a means -- it is characterized by things.

[0019]

[Embodiment of the Invention] Hereafter, the cell culture approach of this invention and the gestalt of one operation of cell culture equipment are explained, referring to an attached drawing.

[0020] As shown in drawing 1 , drawing 2 , and drawing 3 , the cell culture equipment of this invention is first equipped with the container 1. This container 1 is equipped with the cell culture room 11 in which a culture medium is held, an entering means equipped with the upper inlet port 13 for feeding a culture medium, and the bottom inlet port 12, the appearance means 14 for discharging a culture medium, the overflow opening 15 for discharging it, in case a culture medium overflows, etc. The 1st tank 2 is formed in a lower part side from the cell culture room 11, and the 2nd tank 3 is formed in the upper part side.

[0021] In the cell culture room 11, the base material 7 for offering the scaffold for a cell (fixed front face) is formed. this base material 7 -- the voice of this operation -- although it arranges and becomes so that it may set like, the plate-like substrate 71 with which plurality stood straight may be boiled in detail and it may meet, that gestalt is not a ****** thing at the example of illustration. For example, the thing which consists of a fence-like substrate, or the thing which adopts padding as a charge of cell adhesion material is also used.

[0022] In the gestalt of this operation, the circulation means for circulating a culture medium is arranged. This circulation means consists of a supply pipe 41, an exhaust pipe 42, an overflow pipe 43, and the time flow tube 44. The supply pipe 41 is open for free passage with the upper inlet port 12, the bottom inlet port 13, and the 2nd tank 3, and the exhaust pipe 42 is open for free passage with the appearance means 14 and the 1st tank 2, and the overflow pipe 43 has extended between the overflow opening 15 and the 1st tank 2. The time flow tube 44 makes the 1st tank 2 and 2nd tank 3 open for free passage through the pump means 45, and is arranged. That is, a culture medium is transportable to the 2nd tank 3 from the 1st tank 2 with the pump means 45.

[0023] The valves V1, V2, V3, and V31 shown in drawing 1 and drawing 2 are control valves for controlling the flow rate of the culture medium in each of an overflow pipe 43, an exhaust pipe 42, and a supply pipe 41. A valve 31 is a cross valve and, thereby, a culture medium is introduced into the cell culture room 11 via the upper inlet port 12 or the bottom inlet port 13 according to actuation. Filters F1 and F2 are formed for the cell culture room 11, and filters F3 and F4 are formed for the 1st tank 2 and the 2nd tank 3. A fresh air is supplied to the cell culture room 11 by the air pump 5 via a filter F1. Moreover, the level controller 9 for measuring and controlling the level of the culture medium in it is formed in the 1st tank 2. If the culture medium in the 1st tank 2 goes up to predetermined level, this level controller 9 can operate the pump means 45, and can transport a culture medium to the 2nd tank 3.

[0024] The reference mark 6 has pointed out the siphon means and consists of tubing of inverted U typeface. The end section of the siphon means 6 is inserted in the culture

medium in the 1st tank 2, and the other end is connected with the exhaust pipe 42. If the level of the front face of the culture medium in the cell culture room 11 goes up to predetermined level higher than the upper limit marginal height h2 of a substrate 71, the height of this siphon means 6 sucks up a culture medium, and is set as extent transportable to the 1st tank 2 via the siphon means 6.

[0025] In the gestalt of this operation, it has a culture-medium homogeneity distribution means 8 to distribute to homogeneity the upper part of two or more substrates 71 in the cell culture room 11, or the culture medium which is prepared caudad and flows into the cell culture room 11. Since the culture-medium homogeneity distribution means 8 is established up in the example shown in drawing 1 and 2, a culture medium is distributed by that cause and can fall to two or more substrates 71 at homogeneity. This culture-medium homogeneity distribution means 8 consists of a distribution tube 81 with which two or more distribution openings have opened, and that end is connected with the upper inlet port 13, and a tray 82 which drops two or more substrates 71 to homogeneity through two or more openings of said base temporarily in response to the fact that the culture medium which two or more openings are prepared in a base, and falls from distribution opening of a distribution tube 81. Of course, although not shown in drawing, it may replace with a tray 82 and a spray may be used.

[0026] Actuation of the cell culture approach of this invention is begun from a cell adhesion process. In this process, first, a valve V3 is controlled and a culture medium is introduced from the 2nd tank 3 by the predetermined flow rate to the cell culture room 11 by a self-weight or differential pressure. Since it is held while the valve V1 attached in the overflow opening 15 had opened at this time, the culture medium in the cell culture room 11 can be raised to the level beyond h4, and two or more whole substrates 71 can be immersed. The culture medium which overflows from the overflow opening 15 flows into the 1st tank 2 by a self-weight or differential pressure via an overflow pipe 43. The level of the culture medium in the 1st tank 2 is controlled by the level controller 9, and is maintained by predetermined level. That is, a pump can be stopped, if a level controller 9 will operate the pump means 45 if the level in the 1st tank 2 goes up to high predetermined level, a culture medium is transported to the 2nd tank 3 and it falls on low predetermined level. If it does so, after a culture medium passes along the cell culture room 11, the 1st tank 2, and the 2nd tank 3, it can be again circulated through it to the cell culture room 11. Although the time amount of circulation is for about 30 minutes, the condition of cell adhesion can be seen and adjusted.

[0027] If cell adhesion in a substrate 71 is completed, with the valve V3 opened, an overflow valve V1 will be closed and a valve V2 will be opened. Since the level of the culture medium in the cell culture room 11 is much higher than the height of the siphon means 6 at this time, a culture medium is introduced into the 1st tank 2 by siphon operation via the appearance means 14. Therefore, with **, the bottom of steps exposes [the level of the culture medium in the cell culture room 11] the guide-peg scene of a substrate 71, while only the thin film-like culture medium had remained on the field. As mentioned above, it is operated so that it may be immersed in exchange or the guide-peg scene of a substrate 71 may be exposed to a culture medium. Said actuation is suspended after a cell fully covers the front face of a substrate 71. As for actuation of 1 circulation, it is desirable that it is for about 30 minutes. It is going up again from the time of the time amount of 1 circulation, i.e., level, beginning to fall from high level, and the fixed flow

rates f1 and f2 which flow through each of tubing 41 and 43 can determine the time amount of this time of being quantity and returning to level as follows.
[0028] Each of h1 and h2 which are shown in drawing 2 expresses the height from the pars basilaris ossis occipitalis of the cell culture room 11 to the lower limit edge of a substrate 71, and the height from said pars basilaris ossis occipitalis to the upper limit edge of a substrate 71. And h3 is the height of the siphon means 6 from said pars basilaris ossis occipitalis, h4 is the height of the overflow opening 15 from said pars basilaris ossis occipitalis, and it is hx. It is the height of the field of the culture-medium level from said pars basilaris ossis occipitalis. Moreover, W shown in drawing 3 is the width of face (equivalent to the width of face of the cell culture room 11) of a substrate 71, and L is the die length (equivalent to the die length of the cell culture room 11) of the base material 7 which consists of two or more substrates 71. Since a culture medium is fed into the cell culture room 11 and the siphon means 6 does not commit the culture medium in the cell culture room 11 by the height h3 of the siphon means 6 from a low at the time of steps ******, the flow rate f2 of the culture medium which flows through tubing 43 is 0.
[0029] In the case of 0<hx <h1 or h2<hx <h3, the rate of a speedup on culture-medium level is then shown in a bottom type. namely, -- U1=f1/W1 (1)
It comes out.
[0030] In the case of h1<hx <h2, the climbing speed of culture-medium level is shown in a bottom type. namely, -- U1=f1/W (1-Nd) (2)
(-- however, in a formula, N shows the number of substrates and d shows the thickness of each substrate.) -- it is .
[0031] and the time of the siphon means 6 working and culture-medium level falling -- tubing 41 and 43 -- if it is alike, respectively and the existing flow rates f1 and f2 are temporarily built with a constant -- 0<hx -- < -- h1 or h2<hx -- < -- in the case of h3, the fall velocity of culture-medium level is shown in a bottom type. namely, -- U2= (f2-f1) / W1 (3)
It comes out.
[0032] In the case of h1<hx <h2, the fall velocity of culture-medium level is shown in a bottom type. namely, -- U2=(f2-f1)/W (1-Nd) (4)
It comes out.
[0033] Therefore, the time amount which goes up culture-medium level from 0 to h3 is calculated by the bottom formula.
[0034]
t1 =W[(h1+h2+h3)L +(h2-h1)(1-Nd)]/f1 (5)
[0035] The time amount which falls culture-medium level from h3 to 0 is calculated by the bottom formula.
[0036]
t2 =W[(h1+h3-h2)
+(h2-h1)(1-Nd)]/(f2-f1) (6)
[0037] Therefore, it decides on the time amount of 1 circulation of the time of going up again from the time of level beginning to fall from a high level, and returning to this high level by the bottom formula.
[0038]
tp =t1 +t2 (7)
[0039]

[Effect of the Invention] Since according to the cell culture approach of this invention, and cell culture equipment removal of metabolite and exchange of a culture medium can be performed easily, and it is immersed in a culture medium, or the scaffold front face of a cell is exposed by the culture-medium circulator style and supply of the nutrient by the culture medium and supply of a fresh gas can be easily performed so that the gestalt of operation mentioned above may show, it is applicable also to a high density cell. Moreover, since dissolved oxygen required for growth of a cell can fully be supplied by the culture medium, with high shearing force not generated, sudden exfoliation of the cell from a substrate is avoidable. And it is easily controllable, and since it can design thru/or scale up easily, it is applicable to mass production. Moreover, the adhesion surface area of a cell is large, and since the cell from culture-medium material can be exfoliated easily, a production cost can be lowered. In addition, although the gestalt of operation mentioned above controls the level of the front face of a culture medium and this invention is put in practical use, naturally it can also perform putting a base material in practical use by the method which carries out taking-up-and-down actuation.

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] It is drawing showing roughly the configuration of the whole gestalt of one operation of the cell culture equipment of this invention for an anchorage dependency monolayer cell.
[Drawing 2] It is drawing which expands the configuration of the cell culture room shown in drawing 1 , and is shown more in a detail.
[Drawing 3] It is the perspective view expanding and showing the base material in the cell culture room shown in drawing 1 .
[Description of Notations]
12 Upper Inlet Port (Entering Means)
13 Bottom Inlet Port (Entering Means)
14 Appearance Means
2 1st Tank (Circulation Means)
3 2nd Tank (Circulation Means)
V1 Overflow valve
V2 The 1st control valve (circulation means)
V3 The 2nd control valve (circulation means)
5 Gas Supply Means
6 Siphon Means (Circulation Means)
7 Base Material
8 Distribution Means (Circulation Means)
11 Cell Culture Room
12 Upper Inlet Port (Entering Means)
13 Bottom Inlet Port (Entering Means)
14; -- 13 and 12 Receipts-and-payments means
43 Overflow Pipe
44 Time Flow Tube (Circulation Means)

45 Pump Means (Circulation Means)
71 Substrate
h1 Lower limit marginal height
h2 Upper limit marginal height

(19)日本国特許庁（ＪＰ）　(12)公開特許公報（Ａ）　(11)特許出願公開番号

特開平10－191961

(43)公開日　平成10年(1998)７月28日

(51)Int.Cl.[6]	識別記号	ＦＩ	
Ｃ１２Ｍ　3/00		Ｃ１２Ｍ　3/00	Ａ
Ｃ１２Ｎ　5/06		Ｃ１２Ｎ　5/00	Ｅ

審査請求　有　請求項の数12　ＯＬ　（全７頁）

(21)出願番号　特願平9－160316

(22)出願日　平成９年(1997)６月17日

(31)優先権主張番号　８５１０７３４４

(32)優先日　1996年６月18日

(33)優先権主張国　台湾（ＴＷ）

(71)出願人　597085671
廖　明一
台湾、台北市南港区昆陽街161号

(72)発明者　廖　明一
台湾、台北市南港区昆陽街161号

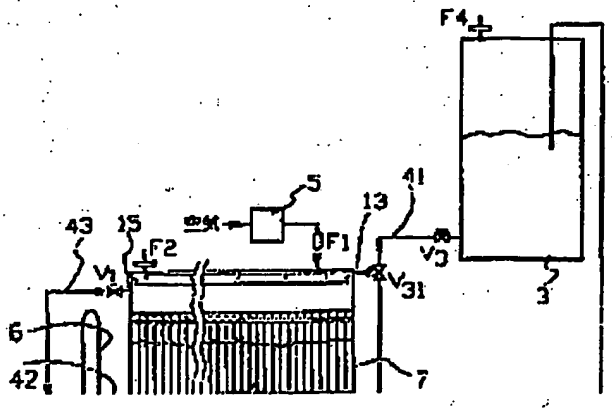
(74)代理人　弁理士　飯江　武彦　（外４名）

(54)【発明の名称】　細胞の培養方法及び培養装置

(57)【要約】

【課題】代謝物除去及び培地交換が容易で、細胞付着基材表面が培地に浸漬または露出され、培地による養素の供給及び新鮮気体の供給が容易で高密度細胞に適用出来、高剪断力が発生せずに培地により細胞成長の為の溶存酸素を十分供給し、細胞基材からの細胞の不意な剥離を避けることができ、制御が容易で生産規模拡大が容易である細胞の培養方法及び培養装置を提供することである。

【解決手段】出入手段１４：１３、１２を有し細胞付着



1

【特許請求の範囲】

【請求項１】　出手段（１４）及び入り手段（１３，１２）を有する細胞培養室（１１）と、細胞培養室（１１）内に設けられている細胞付着用の複数の基材（７）と、出手段（１４）及び入り手段（１３，１２）と連通され培地を出手段（１４）及び入り手段（１３，１２）をへて細胞培養室（１１）に循環させる循環手段（２，３，Ｖ３，Ｖ２，６，８，４４，４５）と、を備えた屈細胞の培養装置において、

前記循環手段が、基材（７）を細胞培養室（１１）に対し動かさない状態で、基材（７）の表面に対する培地のレベルを基材（７）の上端縁高さ（ｈ２）より高いレベルと下端縁高さ（ｈ１）より低いレベルとの間に上げ・下げ操作をし、且つ、

培地のレベルが前記低いレベルに下がると入り手段（１２，１３）を経由して培地を供給し、また、培地のレベルが前記高いレベルに上がると出手段（１４）を経由して培地を排出することができるように構成されている、

ことを特徴とする細胞の培養装置。

【請求項２】　前記循環手段は、出手段（１４）と連通し細胞培養室（１１）の底部より低い所に置かれている第１の貯槽（２）を備えている、

ことを特徴とする請求項１に記載の細胞の培養装置。

【請求項３】　前記循環手段は、細胞培養室（１１）の上流側で細胞培養室（１１）の天井よりも高い位置に置かれている第２の貯槽（３）を備えている、

ことを特徴とする請求項２に記載の細胞の培養装置。

【請求項４】　前記循環手段は、第１の貯槽（２）と第２の貯槽（３）との間に設けられているポンプ手段（４５）を備えている、

ことを特徴とする請求項３に記載の細胞の培養装置。

【請求項５】　前記循環手段は、出手段（１４）及び第１の貯槽（２）の夫々と連通し培地のレベルが前記高いレベルに上がると吸い上げ作用を行なうサイホン手段を備えている、

ことを特徴とする請求項４に記載の細胞の培養装置。

【請求項６】　前記循環手段は、培地の流出量を制御するよう出手段（１４）に連通して設置されている第１の制御弁（Ｖ２）と、培地の流入量を制御するよう入り手段（１２，１３）に連通して設置されている第２の制御

2

ことを特徴とする請求項７に記載の細胞の培養装置。

【請求項９】　前記入り手段は、細胞培養室（１１）の天井に近い箇所に設けられた第１の入口（１３）と、細胞培養室（１１）の底部に近い箇所に設けられた第２の入口（１２）と、を備えている、

ことを特徴とする請求項８に記載の細胞の培養装置。

【請求項１０】　細胞培養室（１１）内の基材（７）の上方または下方に設けられて細胞培養室（１１）に流れ込む培地を均一に分配する培地均一分配手段（８）を更に備えている、

ことを特徴とする請求項９に記載の細胞の培養装置。

【請求項１１】　細胞培養室（１１）の上部から細胞培養室（１１）に気体を供給する気体供給手段（５）を更に備えている、

ことを特徴とする請求項１０に記載の細胞の培養装置。

【請求項１２】　（ａ）．出手段（１４）及び入り手段（１３，１２）と、細胞付着のための基材（７）と、を有する細胞培養室（１１）を設置し；

（ｂ）．細胞培養室（１１）に培地を供給して基材（７）に細胞を付着させ；

（ｃ）．基材（７）の表面に細胞を成長させながら基材（７）を細胞培養室（１１）に対し動かさない状態で、出手段（１４）及び入り手段（１３，１２）を経由して培地を循環させると共に、基材（７）の表面に対する培地のレベルを基材（７）の上端縁高さ（ｈ２）より高いレベルと下端縁高さ（ｈ１）より低いレベルとの間で上げ下げ操作し；

（ｄ）．培地のレベルが前記高いレベルに上がると出手段（１４）を経由して培地を流出させるように制御し；

（ｅ）培地のレベルが前記低いレベルに下がると入り手段（１２，１３）を経由して培地を流入させるように制御する；ことを特徴とする細胞の培養方法。

【発明の詳細な説明】

【０００１】

【発明の属する技術分野】この発明は動物細胞の培養方法及び培養装置に関し、特に、足場依存性単一層細胞の培養方法及び培養装置に関する。

【０００２】

【従来の技術】動物細胞由来製品の需要増加に伴って動物細胞の培養方法の開発が製薬産業の必須不可欠な什亩

enges to Scale-up" The Canadian Journal of Chemical Engineering; "Specialized Techniques" Volume 69, April, 1991, page 409-420"; "Specialized Techniques". Culture of Animal Cells. Chapter 23, pages 371-377]。

【0004】動物細胞培養の過程においては、酸素などの気体及び養素を十分に供給するのがその基本要求である。気体または養素が十分に供給されなければ、細胞の成長が阻害される。そして、代謝物質のレベルの制御も要求される。何故ならば培地における代謝物濃度が高過ぎると細胞の成長が阻害されるからである。

【0005】前記ローラ瓶式はバス型で、培地の循環系統がついていない。この方式は、培地を収容しフレーム上に支承されて転がる瓶を備えている。通常は、この瓶の一つに瓶の内容量の1／10～1／5の培地を入れ、瓶を回転させながら細胞を瓶の内壁に付着させて成長させると共に、回転時は、培地に浸されていない瓶内壁表面に形成される薄膜によって気相と液相との間の気体交換を促進させ、培地中の溶存酸素量を増加させる。従って、この方式によっては、たとえ細胞の密度が高くなっても、酸素など気体の供給を十分に受けることができ、また、瓶内壁への細胞の付着表面積が増加し、且つ、緩やかな撹拌効果がある。そして、この方式においては、ピペットまたはぜん動型ポンプなどの注入装置や分配装置によって新鮮な培地を添加することもできる。

【0006】しかしながら、この方式によっては、pH及び溶存酸素を制御することができない。その上、培地の循環系統がついていないため、培地を頻繁に交換することにより有害性代謝物の排除及び養素の補足をしなければならないので、手間がかかる。そして、量産には多くの瓶をつかわなければならないので、手間がもっとかかり、均一な品質も得難い。従ってその応用範囲には限りがある。

【0007】培地循環系統がついている細胞培養方式は培地の補足及び代謝物の排除に有利である。このような方式においては、細胞は常に培地に浸漬されており、目

滅給方式は、培養密度及び効率が高い方式であるが、小分子量の養素の供給及び代謝物の排除は、通常、膜を隔切っての濃度勾配に依存する拡散によって達成されるので、溶存酸素及び養素は中空繊維の長さや厚さの増加に伴って不足する。従って、中空繊維方式では、やはり細胞の量産には難しい。

【0010】そして、陶磁器方式においては、多孔性セラミックシリンダの通路に細胞が接種されており、且つ、培地が養素を供給しながら代謝物を除去するように前記通路を通過する。この時、溶存酸素量を増加させるには、ポンプで培地を循環させる。しかしながら、十分な溶存酸素量を供給するために培地の循環速度を増加させると、この培地の循環速度の増加により細胞が足場（固定表面）から剥離し生産性に悪影響をきたす可能性がある。

【0011】

【発明が解決しようとする課題】この発明は上記事情の下でなされ、この発明の目的は、代謝物の除去及び培地の交換が容易に行なえ、且つ、細胞の足場表面が培地から浮上し、培地の薄膜と気体との間の気体交換が容易に行なうことが出来て、たとえ高密度細胞においても、酸素不足の問題を解決することができる、細胞の培養方法及び培養装置を提供することである。

【0012】この発明はまた、高剪断力が発生することなく、培地によって細胞の成長のための溶存酸素を十分に供給することができる、細胞の培養方法及び培養装置を提供することを目的としている。

【0013】さらにまた、この発明は、容易に制御でき、且つ、生産規模のスケールアップが容易な、細胞の培養方法及び培養装置を提供することを目的としている。

【0014】さらにこの発明は、細胞の付着表面積が広く、且つ、培養基材からの細胞の剥離を容易に行なえる、細胞の培養方法及び培養装置を提供することを目的としている。

【0015】

【課題を解決するための手段】本願の発明は上記目的を達成するために、この発明の細胞の培養装置では、出手段及び入り手段を有する細胞培養室と、細胞培養室内に設けられている細胞付着用の基材と、出手段及び入り手

【００１６】前記循環手段は、好ましくは、出手段と連通し細胞培養室の底部より低い所に置かれている第１の貯槽と、細胞培養室の上流側で細胞培養室の天井より高い位置に置かれている第２の貯槽と、第１の貯槽と第２の貯槽との間に設けられているポンプ手段と、出手段及び第１の貯槽の夫々と連通し培地のレベルが前記高いレベルに上がると吸い上げ作用を行なうサイホン手段と、培地の流出量を制御するよう出手段に連通して設置されている第１の制御弁と、培地の流入量を制御するよう入り手段に連通して設置されている第２の制御弁と、基材の天辺より高い箇所から細胞培養室と連通したあふれ管と、あふれ管に設けられているあふれ弁と、第１の貯槽中の培地の表面のレベルを測定する培地表面レベル測定手段などを必要に応じて備えている。

【００１７】また、入り手段は、好ましくは、細胞培養室の天井に近い箇所に設けられた第１の入口と、細胞培養室の底部に近い箇所に設けられた第２の入口と、を備えている。そしてまた、本願の細胞の培養装置は、好ましくは、細胞培養室内の基材の上方または下方に設けられて細胞培養室に流れ込む培地を均一に分配する培地均一分配手段と、細胞培養室の上部から細胞培養室に気体を供給する気体供給手段と、を更に備えている。

【００１８】そして、上述した本願の発明の目的を達成するために、本願発明の細胞の培養方法では：（ａ）．出手段及び入り手段と、細胞付着のための基材と、を有する細胞培養室を設置し；（ｂ）．細胞培養室に培地を供給して基材に細胞を付着させ；（ｃ）．基材の表面に細胞を成長させながら基材を細胞培養室に対し動かさない状態で、出手段及び入り手段を経由して培地を循環させると共に、基材の表面に対する培地のレベルを基材の上端縁高さより高いレベルと下端縁高さより低いレベルとの間で上げ下げ操作し；（ｄ）．培地のレベルが前記高いレベルに上がると出手段を経由して培地を流出させるように制御し；培地のレベルが前記低いレベルに下がると入り手段を経由して培地を流入させるように制御する；ことを特徴としている。

【００１９】

【発明の実施の形態】以下．添付の図面を参照しながら本発明の細胞培養方法及び細胞培養装置の１つの実施の形態を説明する。

の基材７は、この実施の態様において．複数の直立した平板状の基板７１を逐一に対面するように配列してなるが．その形態は図示の例に限らるものではない。例えば．柵状の基板からなるもの、または．詰め物を細胞付着用材料として採用するものも使用される。

【００２２】この実施の形態においては、培地を循環させるための循環手段が配設されている。この循環手段は．供給管４１と、排出管４２と、あふれ管４３と、回流管４４とからなる。供給管４１は上入口１２．下入口１３及び第２の貯槽３と連通しており．排出管４２は出手段１４及び第１の貯槽２と連通しており、そして、あふれ管４３はあふれ口１５と第１の貯槽２との間に延在している。回流管４４はポンプ手段４５を介して第１の貯槽２と第２の貯槽３とを連通させて配設されている。即ち．ポンプ手段４５により、培地を第１の貯槽２から第２の貯槽３に移送することができる。

【００２３】図１及び図２に示す弁Ｖ１，Ｖ２．Ｖ３，Ｖ３１は、あふれ管４３．排出管４２及び供給管４１の夫々における培地の流量を制御するための制御弁である。弁３１は三方弁であり．それにより、培地は操作に応じて上入口１２または下入口１３を経由して細胞培養室１１に導入される。フィルターＦ１．Ｆ２は細胞培養室１１のために設けられ．そして．フィルターＦ３，Ｆ４は第１の貯槽２及び第２の貯槽３のために設けられている。フィルターＦ１を経由して新鮮な空気がエアポンプ５によって細胞培養室１１に供給される。また．第１の貯槽２には．その中の培地のレベルを測定し制御するためのレベルコントローラ９が設けられている。第１の貯槽２内における培地が所定のレベルに上がると．このレベルコントローラ９はポンプ手段４５を作動して培地を第２貯槽３へ移送することができる。

【００２４】参照符号６はサイホン手段を指摘しており．倒置したＵ字形の管からなる。サイホン手段６の一端部は第１の貯槽２内の培地に挿入され、他端部は排出管４２と連結されている。このサイホン手段６の高さは．細胞培養室１１における培地の表面のレベルが基板７１の上端縁高さｈ２より高い所定のレベルに上がると．培地を吸い上げて、サイホン手段６を経由し．第１の貯槽２に移送することができる程度に設定されている。

的に受けて前記底面の複数の開口を通って複数の基板71に均一に落下させるトレー82とからなる。もちろん、図に示されていないが、トレー82に代えてスプレーを使用しても良い。

【0026】本発明の細胞培養方法の操作は細胞付着工程から始める。この工程においては、まず、弁V3を制御し、自重または差圧により、培地を第2の貯槽3から細胞培養室11へ所定流量で導入する。この時、あふれ口15に取り付けられている弁V1が開けられたまま保持されるので、細胞培養室11内における培地をh4以上のレベルに上げて複数の基板71の全体を浸漬することができる。あふれ口15から溢れてくる培地はあふれ管43を経由して自重または差圧により第1の貯槽2に流れ込む。第1の貯槽2における培地のレベルはレベルコントローラ9に制御されて所定のレベルに維持されている。即ち、第1の貯槽2におけるレベルが所定の高いレベルに上がると、レベルコントローラ9はポンプ手段45を作動して培地を第2の貯槽3に移送し、また、所定の低いレベルに下がると、ポンプを停止させることができる。そうすると、培地は細胞培養室11，第1の貯槽2，そして第2の貯槽3を通ってから再び細胞培養室11へと循環することができる。循環の時間は約30分間であるが、細胞付着の具合をみて調整することができる。

【0027】基板71への細胞付着が完成すれば、弁V3を開けたまま、あふれ弁V1を閉じ、弁V2を開ける。この時、細胞培養室11内の培地のレベルはサイホン手段6の高さよりずっと高いので、培地はサイホン作*

$$U1 = f1 / W1$$

である。

【0030】h1<h，<h2の場合，培地レベルの上※

$$U1 = f1 / W(1-Nd)$$

（ただし、式中で、Nは基板数を示し、dは各基板の厚さを示す。）である。

【0031】そして、サイホン手段6が働いて培地レベルが下がる時，管41及び43それぞれにある流量f ★

$$U2 = (f2-f1) / W1$$

である。

【0032】h1<h，<h2の場合，培地レベルの降☆

$$U2 = (f2-f1) / W(1-Nd)$$

*用により、出手段14を経由して第1の貯槽2に導入される。そのため、細胞培養室11内にある培地のレベルが段々下がると共に、基板71の足場面は、面上に薄膜状の培地だけが残ったまま露出する。上記のように、基板71の足場面を培地に対して交換的に浸漬したり露出したりするように操作する。細胞が基板71の表面を十分に被覆した後に前記操作を停止する。1循環の操作は約30分間であることが好ましい。1循環の時間，即ちレベルが高いレベルから下がり始める時点から再び上がって該高いレベルに戻った時点までの時間は、下記のように，管41及び43のそれぞれを通って流れる一定の流量f1及びf2によって決定することができる。

【0028】図2に示すh1及びh2のそれぞれは細胞培養室11の底部から基板71の下端縁までの高さ、及び，前記底部から基板71の上端縁までの高さ，を表わす。そして、h3は前記底部からのサイホン手段6の高さであり、h4は前記底部からのあふれ口15の高さであり，h，は前記底部からの培地レベルの面の高さである。また、図3に示すWは基板71の幅（細胞培養室11の幅に相当）であり、Lは複数の基板71からなる基材7の長さ（細胞培養室11の長さに相当）である。培地を細胞培養室11に送入して細胞培養室11内の培地を低レベルからサイホン手段6の高さh3までに段々上げる時、サイホン手段6が働かないので、管43を通って流れる培地の流量f2は0である。

【0029】その時，0<h，<h1またはh2<h，<h3の場合，培地レベルの上昇速率が下式に示される。即ち，

(1)

※昇速度が下式に示される。即ち，

(2)

★1，f2を高数と仮設すれば、0<h，<h1またはh2<h，<h3の場合、培地レベルの降下速度が下式に示される。即ち，

(3)

☆下速度が下式に示される。即ち，

(4)

$$t_0 = t_1 + t_2$$

(7)

【0039】

【発明の効果】上述した実施の形態から分かるように、この発明の細胞培養方法及び細胞培養装置によれば、培地循環機構により、代謝物の除去及び培地の交換が容易に行なえ、且つ、細胞の足場表面が培地に浸漬されたり露出されたりし、培地による養素の供給及び新鮮な気体の供給が容易に行なえるので、高密度細胞にも適用することができる。また、高剪断力を発生させないまま培地によって細胞の成長に必要な溶存酸素を十分に供給することができるので、基板からの細胞の不意な剥離を避けることができる。そして、容易に制御でき、且つ、容易に設計ないしスケールアップすることができるので、量産に適用出来る。また、細胞の付着表面積が広く、且つ、培養基材からの細胞の剥離が容易に行なえるので生産コストを下げることができる。なお、上述した実施の形態は、培地の表面のレベルを制御して本発明を実用化するが、基材を上げ下げ操作する方式で実用化することも当然できる。

【図面の簡単な説明】

【図1】足場依存性単一層細胞の為の本発明の細胞培養装置の1つの実施の形態の全体の構成を概略的に示す図である。

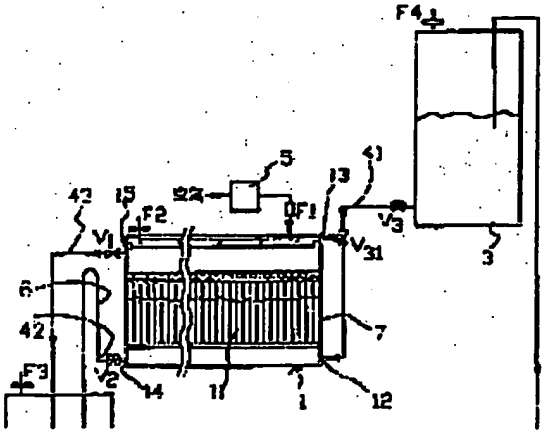
【図2】図1に示されている細胞培養室の構成を拡大してより詳細に示す図である。

【図3】図1に示されている細胞培養室中の基材を拡大＊して示す斜視図である。

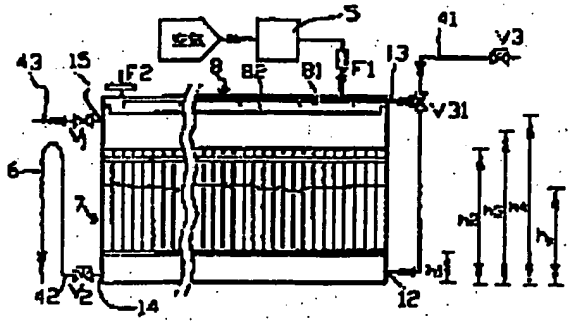
【符号の説明】

12　上入口（入り手段）
13　下入口（入り手段）
14　出手段
2　第1の貯槽（循環手段）
3　第2の貯槽（循環手段）
V1　あふれ弁
V2　第1の制御弁（循環手段）
V3　第2の制御弁（循環手段）
5　気体供給手段
6　サイホン手段（循環手段）
7　基材
8　分配手段（循環手段）
11　細胞培養室
12　上入口（入り手段）
13　下入口（入り手段）
14；13，12　出入り手段
43　あふれ管
44　回流管（循環手段）
45　ポンプ手段（循環手段）
71　基板
h1　下端縁高さ
h2　上端縁高さ

【図1】



【図2】



【図3】